



Memorandum

Date: March 22, 2012
From: Foods Program Science and Research Steering Committee
Subject: Guidelines for the Validation of Chemical Methods for the FDA Foods Program
To: Foods Program Executive Committee


The FDA Foods Program Science and Research Steering Committee (SRSC), made up of representatives from the Office of Foods, the Center for Food Safety and Applied Nutrition, the Center for Veterinary Medicine, the Office of Regulatory Affairs, the National Center for Toxicological Research, the Office of International Programs, and the Office of the Chief Scientist, is charged with the task of prioritizing, coordinating and integrating food- and feed-related science and research activities across the operating units of FDA's Foods Program.

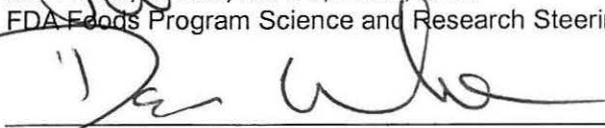
As a regulatory agency tasked with ensuring the safety of the nation's food supply, it is imperative that the laboratory methods needed to support regulatory compliance, investigations and enforcement actions meet the highest analytical performance standards appropriate for their intended purposes. Development of standardized validation requirements for all regulatory methods used to detect chemical and radiological contaminants, as well as microbial pathogens, used in our laboratories is a critical step in ensuring that we continue to meet the highest standards possible.

The attached document, now formally adopted by the SRSC, establishes those requirements that must be fulfilled in the evaluation of chemical methods to be used in our testing laboratories. In the near future, these guidelines will be posted on FDA's website and additional venues for publication and dissemination of these guidelines are being explored and will be announced when they become available. Please share this chemical methods validation standard operating procedure with anyone who may be conducting or supervising chemical methods validation projects or otherwise needs to be aware of these new requirements.

Shortly, a method validation sub-committee will be constituted and charged with providing guidance and oversight to all validation studies. In the interim, all inquiries pertaining to method validation for chemical contaminants should be addressed directly to your representative on the SRSC.

Thank you,



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US Food and Drug Administration Office of Foods

Guidelines for the Validation of Chemical Methods for the FDA Foods Program

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I. INTRODUCTION

Purpose

The U.S. Food and Drug Administration (FDA) is responsible for ensuring the safety of roughly 80% of the nation's food supply. FDA laboratories contribute to this mission through routine surveillance programs, targeted regulatory analyses, and emergency response when contaminated food or feed is detected or suspected in a public health incident. The effectiveness of these activities is highly dependent on the quality and performance of the laboratory methods needed to support regulatory compliance, investigations and enforcement actions. To ensure that the chemical methods employed for the analysis of foods meet the highest analytical performance standards appropriate for their intended purposes, FDA's Office of Foods has established criteria by which all Agency-wide regulatory methods designed to detect chemicals in foods should be evaluated and validated. This document defines four standard levels of performance for use in the validation of analytical regulatory methods for chemical analytes in foods.

Authority

All criteria established in this document for analytical method validation have been adopted and approved by the Office of Foods (OF) and the Science and Research Steering Committee (SRSC). Upon enactment of these guidelines, the SRSC will authorize the formation of a Methods Validation Subcommittee (MVS) to serve as the governing body for all method validation concerns. Procedures for the MVS' operations have not yet been developed.

Scope

These criteria apply to FDA laboratories as they develop and participate in the validation of analytical regulatory methods for chemical analytes in anticipation of Agency-wide Foods Program implementation. These criteria do not apply to methods developed by or submitted to FDA under a codified process or official guidance (e.g., in the Code of Federal Regulations, CPGs, etc.) such as for veterinary drug approval. For such studies, the appropriate CVM or other Program guidance documents should be followed. Once a validated method is established, it can be utilized by other laboratories after being subjected to method verification. However, method verification is normally part of a local laboratory's quality control procedures and is not considered within the scope of this validation document.

General Responsibility of the Originating Laboratory

It is the responsibility of the originating (developing) laboratory to ensure proper adherence to all criteria described in this document. The originating laboratory should work in consultation with the MVS or its designated method validation advisory panel throughout the validation process.

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Overview of Method Validation

Method validation is the process of demonstrating or confirming that a method is suitable for its intended purpose. The purpose of these methods may include but is not limited to qualitative analysis, quantitative analysis, screening analysis, confirmatory analysis, limit tests, matrix extensions, platform extensions, and emergency/contingency operations. Validation includes demonstrating performance characteristics such as accuracy, precision, sensitivity, selectivity, limit of detection, limit of quantitation, linearity, range, and ruggedness to ensure that results are meaningful and appropriate to make a decision. Method validation is a distinct phase from method development/optimization and should be performed *subsequent* to method development. Methods may be validated for one or more analytes, one or more matrices, and one or more instruments or platforms. The method developer validates a method by conducting experiments to determine the specific performance characteristics that serve to define and quantify method performance.

Applicability

This document establishes validation criteria for regulatory methods that are to be widely used to detect chemical analytes in food and FDA regulated products covered by the Foods Program including, but not limited to, the following:

- Intentional Adulterants/Poisons
- Persistent Organic Pollutants
- Pesticides
- Chemotherapeutic Residues
- Color Additives
- Food Additives and Preservatives
- Decomposition Products
- Dietary Supplement Ingredients/Adulterants
- Food Allergens
- Mycotoxins
- Seafood and plant toxins
- Nutrients
- Toxic Elements
- Veterinary Drug Residues

These validation guidelines are not applicable to situations when the method is being extended to handle what is likely to be a very limited (perhaps one time) use by one laboratory and is therefore not intended for Agency-wide regulatory use. For example, when a single pesticide laboratory receives several new food matrices for multiresidue analyses that were not covered in previous validation of the method, these guidelines would not generally be required and a more abbreviated validation/verification within the pesticide program's guidelines may be acceptable.

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Requirements

Method validation is required for:

- Submission of a new or original method.
- Expansion of the scope of an existing method to include additional analytes.
- Expansion of the scope of an existing method to include additional matrices.
- Changes in the intended use of an existing method (*e.g.*, screening vs. confirmatory).
- Modifications to a method that may alter its performance specifications (*e.g.*, changes to the fundamental science of an existing method, significant changes to reagents, apparatus, instrumental parameters, sample preparation and/or extraction, or modification of a method's range beyond validated levels.)

II. CRITERIA AND GUIDANCE FOR THE VALIDATION OF CHEMICAL METHODS

General Validation Tools and Protocol Guidance

The following provides guidelines/tools that should be used to define method performance:

General Protocol: Prepare and analyze method blanks, matrix blanks, reference materials (if available) and matrix spikes (using matrix blanks if available) of known concentration as generally described under the Methods Validation Levels section and Table 1 below. Accuracy or bias and precision are calculated from these results. Data will also be used to evaluate matrix effects and ruggedness/robustness of the method resulting from changes in the sample matrix.

The following general validation tools should be used to generate method performance characteristics as described in the Performance Characteristics section below.

Blanks: Use of various types of blanks enables assessment of how much of the result is attributable to the analyte in relation to other sources. Analyze blanks and compare these results to the limit of detection.

Reference materials and certified reference materials: The use of known reference materials (when available and applicable) can be incorporated to assess the accuracy or bias of the method, as well as for obtaining information on interferences.

Matrix Blank: A substance that closely matches the samples being analyzed with regard to matrix components. Matrix blanks are used to establish background level (presence or absence) of analyte(s) and to verify that sample matrix and equipment used does not interfere with or affect analytical signal.

Matrix Spikes (Laboratory Fortified Matrix): Recovery determinations can be estimated from fortification or spiking with a known amount of analyte and calculation of spike recoveries. (Note: spike recovery may not be truly representative of recovery from naturally incurred analytes.) Matrix effects can also be assessed with these samples. Accuracy or bias and precision are calculated from these results. The data can also be used to evaluate robustness of the method resulting from changes in the sample matrix.

Incurred Samples: Samples that contain (not laboratory fortified) the analyte(s) of interest (if available) may also be used to evaluate precision and bias (if analyte concentration(s) are reliably known). Analyte recovery can also be evaluated through successive extractions of the sample and/or comparison to another analytical procedure with known bias.

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Reagent Blank: Incorporates all reagents used in the method and is subjected to all sample processing operations. Serves to verify that reagents are analyte free and equipment used does not interfere or affect analytical signal.

Replicate Analyses: Precision of the analytical process can be evaluated using replicate analyses. The originating laboratory should assure that adequate sample replicates are performed and that results from replicate measurements of each analyte are compared. Minimally, the method repeatability should be evaluated.

Interferences: Spectral, physical, and chemical interferences can be evaluated by analyzing samples containing various suspected interferences. Carryover can be evaluated using the incorporation of blanks immediately following standards and samples.

Statistics: Statistical techniques are employed to evaluate accuracy, trueness (or bias) precision, linear range, limits of detection and quantification, and measurement uncertainty.

Reference Method

A reference method is a method by which the performance of an alternate or new method may be measured or evaluated. For chemical analytes, an appropriate reference method is not always identifiable or available. However, there are some instances in which the use of a reference method is appropriate such as when replacing a method specified for use in a compliance program. Consultation between the originating laboratory and the MVS and the Program Office is suggested when deciding if the use of a reference method will be necessary.

Performance Characteristics

Performance characteristics that should be evaluated in order to validate a method will vary depending on the intended use of the method, the type of method (e.g., quantitative vs. qualitative), and the degree to which it has been previously validated (e.g., matrix extension, analyte extension, platform extension). Although definitions of these characteristics are included in Appendix 1, this document is not meant to address the various ways of calculating characteristics such as method detection level, limit of detection or limit of quantitation.

Performance Characteristics for Validation of New Quantitative Methods: Validation of new quantitative methods should include at a minimum evaluation of the following performance characteristics: accuracy, precision, selectivity, limit of detection, limit of quantitation, linearity (or other calibration model), range, uncertainty, and ruggedness.

Performance Characteristics for Validation of New Qualitative Methods: Validation of new qualitative methods should include at a minimum evaluation of the following

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performance characteristics: sensitivity, selectivity, false positive rate, false negative rate, minimum detectable concentration, ruggedness, and confirmation of identity.

Performance Characteristics for Validation of Method Extensions: Validating the extension of methods that have previously been validated requires a close look at the intended purpose of the extension. In cases where the sample preparation and/or the extraction procedure/analytical method is modified from the existing test procedure, the new method should demonstrate that the modifications do not adversely affect the precision and accuracy of the data obtained. In order to implement the modified method, generally the standard or existing method is first performed. The modified method is then verified against the original method.

Confirmation of Identity

Confirmation of identity for each analyte must be performed as part of the method validation for regulatory enforcement. Unambiguous confirmation of identity usually requires analytically identifying key features of each analyte in the scope of the new method being validated such as with mass spectral fragmentation patterns or by demonstration of results in agreement with those obtained using an independent orthogonal analysis.

FDA has issued guidance documents on the development, evaluation, and application of mass spectrometric methods for confirming the identity of target analytes including: 1) CVM Guidance for Industry 118: Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues and 2) ORA LAB.10: ORA-WIDE PROCEDURE - Guidance for the Analysis and Documentation to Support Regulatory Action on Pesticide Residues. These documents may be consulted when validating mass spectrometric methods for regulatory use.

Method Validation Levels

The following describes the four standard levels of performance defined for method validation of analytical regulatory methods for chemical analytes in foods. Key validation parameters for each level are summarized in Table 1. It is the responsibility of the originating (developing) laboratory in consultation with the MVS to determine the appropriate level of validation required.

NOTE: *Not all methods will or should be validated to the highest level.*

Level One

This is a single laboratory validation level with the lowest level of validation requirements and is limited to emergency use. Performance of the method at this initial level of scrutiny will determine, in part, whether further validation is useful or warranted.

Intended Use: Emergency use/matrix, analyte or platform extension. For example, application of a method developed for a specific analyte(s) to a matrix, not previously validated in response to a real or perceived threat to food safety or

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public health. Validation of method performance with a new matrix is intended to assure that the new matrix will produce accurate and reliable results for all the analytes in the scope of the method. Generally, all targeted analytes still must be included in matrix spikes at this level, if widespread use in this matrix is anticipated for regulatory purposes. As the first level of validation of methods for matrix, analyte or platform extension/emergency use, it would be expected that a more rigorous single laboratory validation at least equivalent to Level Two below would be performed before more widespread non-emergency regulatory use.

Level Two

This is a single laboratory validation level. The originating lab has conducted a comprehensive validation study, with performance criteria similar to an AOAC Single Laboratory Validation study. (If appropriate, a comparison with an existing reference method has been performed.) Some of the criteria of the study may be at a lower level than the AOAC Single Laboratory Validation study, but are appropriate for the developing method at this stage.

Intended Use: Routine regulatory testing, emergency needs, minor method modifications, analyte and matrix extensions of screening methods. If a method validated at this level is expected to have use that is widespread, long term, of high public visibility or potentially involved in international trade conflicts, its validation should be extended to at least Level Three below.

Level Three

This is a multi-laboratory validation level. Level Three validation employs a minimum of one collaborating laboratory in addition to the originating laboratory. Most of the criteria followed by the originating lab are at a level similar to the AOAC full collaborative study level (with comparison to an existing reference method when available and appropriate). The additional collaborating laboratories follow many of the criteria found in an AOAC collaborative study. The main differences are that Level Three validation employs at least one additional collaborating laboratory instead of the eight to ten used by AOAC and requires fewer replicates for each food matrix/spike level.

Intended Use: Methods validated to this level of scrutiny are acceptable for use in all regulatory circumstances including screening analyses, confirmatory analyses, regulatory surveys, and compliance support. If the method is expected to have use that is widespread, long term, of high public visibility or involved in international trade conflicts, it may be appropriate to have its validation extended to Level Four.

Level Four

This validation level has criteria equivalent to a full AOAC Collaborative Study. Any method reaching this level of validation should be able to be submitted for adoption by the AOAC as a fully collaborated method.

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Acceptability Criteria

There are various acceptability ranges for method validation performance criteria that may be appropriate depending on the application or intended use of the methodology and especially the levels of concern, action levels or tolerance for the chemical analyte. Some examples of acceptability ranges used by various national and international organizations and their sources are provided in Appendix 2. Acceptable spike recoveries vary with analyte concentration as indicated in Appendix 2 (e.g., recoveries may fall in approximately the 80-120% range for quantitative methods at the 1 ug/g (ppm) concentration). Repeatability and reproducibility also vary with analyte concentration. The acceptability ranges in Appendix 2 provide approximate target ranges for method developers and the MVS and are not rigid binding guidelines. It is recognized that for some situations such as with difficult matrices, extremely low analyte concentrations (e.g., chlorinated dioxins, persistent organic pollutants), multiresidue methods and with emergency situations these general acceptability ranges may not be achievable or required.

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Table 1. Foods Program Key Validation Parameter Requirements for Chemical Methods*

	Level One: Emergency Use	Level Two: Single Laboratory Validation	Level Three: Multi- Laboratory Validation	Level Four: Full Collaborative Study
Number participating labs	1	1	≥ 2	8 (quantitative) 10 (qualitative)
Number of matrix sources per matrix**	≥1	≥3 recommended	≥3 recommended	≥3 recommended
Number of analyte(s) spike levels per matrix source	≥2 spike levels + 1 matrix blank	≥3 spike levels + 1 matrix blank	≥3 spike levels + 1 matrix blank	≥3 spike levels + 1 matrix blank
Replicates required per matrix source at each level tested per laboratory	≥2 (quantitative) ≥2 (qualitative)	≥2 (quantitative) ≥3 (qualitative)	≥2 (quantitative) ≥3 (qualitative)	≥2 (quantitative) ≥3 (qualitative)
Replicates required at each level tested per laboratory if only one matrix source used	≥4 (quantitative) ≥6 (qualitative)	≥6 (quantitative) ≥9 (qualitative)	≥3 (quantitative) ≥6 (qualitative)	≥2 (quantitative) ≥6 (qualitative)

* References: Adapted from Food Emergency Response Network (FERN), SOP No: FERN-ADM.0008.00, FERN Validation Guidelines for FERN Chemical, Microbiological, and Radiological Methods; and the draft AOAC International, “Standard Method Performance Requirement (SMPR) Documents” (Version 12.1; 31-Jan-11).

**If a variety of food matrices with differing physical and chemical properties are selected, the number of sources for each food sample matrix may be one or more, but if only one food matrix is studied then ≥3 sources are recommended. However, the number of matrix sources may be reduced, particularly if it is difficult to obtain blank matrix sources, as long as the total number of spike levels and matrix combinations are sufficient for adequate statistical evaluation.

III. ADDITIONAL PROCEDURAL GUIDANCE

In addition to the criteria described above in Table 1 for standard quantitative and qualitative methods, additional guidance is provided in this section for specific types of methods or validation situations.

Platform/Instrumentation Extension

Expanding the use of a validated method to include another significantly different instrument or platform requires further validation. Such instances include the use of an instrument or platform similar in scope and function to that currently validated and approved for use; however, it may have major differences in configuration, or detection scheme.

Platform extension validation should generally be performed using Table 1, Level 2 as a guide and can either be performed as a brand new method validation or may be done by comparing the proposed new platform to the platform used in the reference method. In planning platform extension validation, one must determine what degree of cross-correlation between the results obtained on the two platforms will be acceptable.

Examples:

Method A is a validated method for the screening of pesticides on a gas chromatograph coupled to a single quadrupole mass spectrometer (GC-MSD). Gas chromatography coupled to a triple quadrupole mass spectrometer (GC-QQQ), offers certain advantages over the GC-MSD platform in terms of sensitivity, selectivity and scope. In this instance, a comparative method extension validation would be indicated to ensure equivalent results, but if new analytes are added to the scope of the method via the use of the new platform, a brand new method validation would be indicated for the GC-QQQ method.

Method Z is a validated method for the screening of polycyclic aromatic hydrocarbons in seafood using liquid chromatography with a fluorescence detector (LC-FLD). A laboratory would like to transfer this method to a liquid chromatography system that utilizes only a diode-array detector (LC-DAD). In this instance, a comparative method extension validation would be indicated to ensure that the new detection system produces equivalent results to the originally validated method.

Food Matrix Extension

The validation of method performance with a new matrix is intended to assure that the method will continue to produce accurate and reliable results. Emergency matrix extensions (Level 1 in Table1) are intended for those instances in which a validated method is used with a matrix not previously validated in response to a real or perceived threat to food safety or public health and in this type of urgent situation it is not expected that the MVS would be consulted. Matrix extensions of validated methods which are intended to increase the regulatory scope and applicability on a recurring basis would

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minimally fall under Level 2 validation in Table 1. This section provides guidance to extend validated methods to matrices in anticipation that these food commodities will be included in Agency-wide testing. Method developers may wish to consult with the MVS before initiating any Level 2 validation work on matrix extension.

It is generally assumed that the more closely related a new food matrix is to a previously validated matrix for a defined analyte, the greater the probability that the new matrix will behave similarly. It is also usually the case that the regulatory chemical methods employed by FDA are used to analyze a diversity of products representing a large spectrum of matrices. It becomes unfeasible to carry out a matrix extension validation for each single matrix in order to expand the scope of the method. A more reasonable approach to demonstrate the applicability of a method to a set of product matrices is to validate the method for different “categories” of products. For instance, a multi-residue pesticide method can be validated for “high-sugar”, “high-fat”, “high-water”, “dry” and “high-protein” matrices. Appendix 4 provides guidance on commodity categories and gives examples of representative matrices in each category.

The number of different food categories to be validated depends on the applicability and intended use of the method. If the method is specific to only one category, only one type of food need be included. If the applicability is wider (e.g., detection of phthalates in processed foods), then an appropriate number of food categories should be included to represent all anticipated matrices. Depending on how many categories will be validated, a minimum of 1 – 3 representative matrices from each category should be selected.

Limit Tests (common semi-quantitative screening method)

One specific category of qualitative methods includes limit tests (binary or pass/fail tests) for analytes that have a defined level of concern. The purpose of these screening methods is to determine if analyte is present with a concentration near or above the level of concern. This is in contrast to screening methods whose intended purpose is to determine the presence or absence of an analyte at any level. Limit test method validations must include determination of the precision of the method for an analyte(s) at the level of concern.

Limit test screening methods, in general, should avoid false negatives with false negative rates representing less than 5% of the analytical results. The occurrence of false positives is less critical since presumptive positives are further analyzed by quantitative or confirmatory methods. However, false positive rates should typically be less than 10-15% to avoid unnecessary confirmatory testing. Ideally, limit tests are capable of rapidly screening a large number of samples to minimize the need for additional analysis. A common approach used in limit test screening methods is to use a confidence interval to set a laboratory threshold or cut-off value whereby only responses above that value require further testing. For a limit test based on an instrument response, a threshold or cut-off value can be determined by measuring the standard deviation of the response or concentration of an analyte in samples fortified with the analyte at the level of concern.

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Example:

Milk samples (n=21) were fortified with sulfamethazine at the level of concern (10 ng/mL). A LC-MS/MS limit test screening method was used to measure this drug in the extracted milk samples. The mean concentration found was 10.99 ng/mL with a standard deviation of 2.19. A threshold or cut-off value was calculated so that 95% of samples containing sulfamethazine at or above 10 ng/mL would have a response above the threshold value:

$$\begin{aligned}\text{Threshold value} &= [\text{mean concentration} - (t * \text{standard deviation})] \\ &= [10.99 - (1.725 * 2.19)] = 7.21 \text{ ng/mL}\end{aligned}$$

Where t = one-tailed Student's t value for $n-1$ degrees of freedom at the 95% confidence level

This approach can also be used for immunosorbent assays such as enzyme linked immunosorbent assay (ELISA) or optical biosensor assays. These tests may be non-competitive (direct measurement of analyte response) or competitive (indirect measurement). Analysis of data from a competitive immunosorbent test would need to account for the fact that the observed response decreases with increasing analyte concentration; therefore, a response lower than the threshold or cut-off would be considered presumptive positive. For immunosorbent assays, it is also important to measure the response observed for blank matrix samples and to distinguish that the blank response is statistically different from that of the threshold.

Performance characteristics of limit tests:

Validation of new limit tests should include, at a minimum, evaluation of the following performance characteristics: sensitivity, specificity, precision, threshold or cut-off value, false positive rate, false negative rate, minimum detectable concentration (should be lower than threshold/cut-off), ruggedness/robustness.

Qualitative Broad-band (non-targeted) Analyte Screening

Broad-band methods that can detect many compounds are being utilized more frequently as an initial screening step as part of chemical contaminant testing in FDA laboratories. These methods usually involve mass spectrometric analyses and provide qualitative information. For example, the data obtained may be compared to an established reference such as a database of compounds with exact mass and molecular formula information or spectra in a compiled library. For regulatory action, any positive findings from this screen would be confirmed by a targeted method (for example using a LC-MS/MS or GC-MS/MS platform).

Typically, initial validation of these methods is performed using a limited set of representative analytes and representative matrices. For example, sets of analytes that contain compounds from a variety of chemical classes from the area of interest (e.g. pesticides, veterinary drug residues, or common chemical toxins) are tested with the method using representative matrices. The performance characteristics that may be

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evaluated include: sensitivity, selectivity, false positive rate, false negative rate, minimum detectable concentration, ruggedness, and confirmation of identity. It is understood that the method performance may vary with the different classes of compounds, but it is important to have an initial evaluation of the method's capabilities.

Laboratories continuously expand the scope of these broad-band methods by adding new analytes that come to their attention through various sources of intelligence. In addition, a new compound might be found in a sample after acquired data is compared to the reference databases. In these cases, some verification that the analyte can reliably be detected by the screening method is required. When a new compound is added to the scope of a qualitative method, it should first be determined whether this compound belongs to a class of compounds that has already been validated for the broad-band method. If the new compound shares chemical characteristics with an existing class of compounds in the scope of the method, then it may suffice as a partial validation to select a few representative matrices, perform a single level spike in these representative matrices in duplicate and look for partial recovery (any amount recovered is acceptable) in order to assess whether the analyte can be effectively detected by the method.

Another situation that would not typically call for a full-blown validation protocol is when the new analyte being added to the broad-band method is in the scope of another validated method provided that the two methods share the same extraction scheme. In those cases, the new analytes should be able to be "grand-fathered" in to the scope of the broad-band method with minimal additional work, such as a single level spike in representative matrices in duplicate.

Scenarios that may require a full validation would include a new analyte being added to the scope of the broad-band method that was not represented by any of the compound classes already in the scope. Also if the new analyte requires modifications in the extraction protocol due to its chemical characteristics, then its inclusion in the scope should be fully validated as recommended by this guidance. Similarly, if the broad-band method is to be used for a unique matrix that has not been tested by any other validated multi-residue method sharing the same extraction protocol as the broad-band method, then a full validation needs to be conducted to determine if the screening method is appropriate for this new matrix. Additional validation or verification may also be required for a major change in the instrumentation utilized.

Although positive findings by the broad-band method are subjected to confirmatory testing using a targeted method, it is still important to determine, through proper validation and verification protocols, that the broad-band method does not give rise to a high number of false negative findings. False negative in this context means the method fails to detect a residue in its scope when the residue is present in the matrix at or above the minimum detectable concentration. While the positive finding by the broad-band method is subjected to further analysis and scrutiny, negative findings are upheld

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as such and a regulatory decision is made based on these results, *e.g.*, to release the products into commerce.

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IV. REFERENCES AND SUPPORTING DOCUMENTS

AOAC International Method Validation Programs.

<http://www.aoac.org/vmeth/omamannual/omamannual.htm>

AOAC International, "AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals".

http://www.aoac.org/Official_Methods/slv_guidelines.pdf

AOAC International, "Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis".

http://www.aoac.org/Official_Methods/Collaborative_Study_Validation_Guidelines.pdf

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<http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM273418.pdf>

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APPENDIX 1 - Definition of Terms

Accuracy: A measure of the agreement between a test result generated by a specific procedure to the accepted true value, and includes a combination of precision and bias.

Action level: Level of concern for an analyte that must be reliably identified or quantified in a sample.

Analyte: Component measured and/or identified in a test sample by the method of analysis.

Analytical batch: An analytical batch consists of samples which are analyzed together with the same method sequence and same lots of reagents and with the manipulations common to each sample within the same time period (usually within one day) or in continuous sequential time periods.

Bias: The difference between the expectation of the test results and an accepted reference value. Bias is the total systematic error, and there may be one or more systematic error components contributing to the bias.

Blank: A substance that does not contain the analytes of interest and is subjected to the usual measurement process. Blanks can be further classified as method blanks, matrix blanks, reagent blanks, instrument blanks, and field blanks.

Calibration: The set of operations which establish, under specific conditions, the relationship between values of quantities by a measuring instrument or measuring system, or values represented by a material measure or a reference material, and the corresponding values realized by standards.

Calibration Standard: A substance used to calibrate the measuring instrument or system.

Carryover: Residual analyte from a previous sample or standard which is retained in the analytical system and measured in subsequent samples. Also called *memory*.

Certified Reference Material (CRM): Reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes metrological traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence. Note: Standard Reference Material (SRM) is the trademark name of CRMs produced and distributed by the National Institute of Standards and Technology (NIST).

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Check Analysis: Result from a second independent analysis that is found in agreement with the initial or screening analysis. Typically, check analyses are performed by a different analyst using the same method.

Confirmation of Identity: Unambiguous identification of an analyte(s) by a highly specific technique such as mass spectrometry or by demonstration of results from two or more independent orthogonal analyses in agreement.

Confirmatory Analysis: Result from a second independent analysis that is found in agreement with the initial or screening analysis. A different method is often used in confirmation of screening results.

Cut-off Concentration: In qualitative analysis, the concentration of the analyte that is either statistically lower than the level of concern (for limit tests) or at which positive identification ceases (for confirmation of identity methods). See also *Threshold Value*.

False Negative Rate: In qualitative analysis, a measure of how often a test result indicates that an analyte is not present, when, in fact, it is present or, is present in an amount greater than a threshold or designated cut-off concentration.

False Positive Rate: In qualitative analysis, a measure of how often a test result indicates that an analyte is present, when, in fact, it is not present or, is present in an amount less than a threshold or designated cut-off concentration.

Incurred Samples: Samples that contain the analyte(s) of interest, which were not derived from laboratory fortification but from sources such as exogenous exposure or endogenous origin. Examples of exogenous exposure include, for example, pesticide use, consumption by an animal, or environmental exposure.

Interference: A positive or negative response or effect on response produced by a substance other than the analyte. Includes spectral, physical, and chemical interferences which result in a less certain or accurate measurement of the analyte.

Intermediate Precision: Within-laboratory precision obtained under variable conditions, e.g., different days, different analysts, and/or different instrumentation.

Internal Standard: A chemical added to the sample, in known quantity, at a specified stage in the analysis to facilitate quantitation of the analyte. Internal standards are used to correct for matrix effects, incomplete spike recoveries, etc. Analyte concentration is deduced from its response relative to that produced by the internal standard. The internal standard should have similar physico-chemical properties to those of the analyte.

Laboratory Fortified Matrix: See *Matrix Spike*.

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Level of Concern: Level of concern is the concentration of an analyte in a sample that has to be exceeded before the sample can be considered violative. This concentration can be a regulatory tolerance/safe level or a laboratory performance level.

Limit of Detection (LOD): The minimum mass or concentration of analyte that can be detected with acceptable certainty, though not quantifiable with acceptable precision. The term is usually restricted to the response of the detection system and is often referred to as the *Detection Limit*. When applied to the complete analytical method it is often referred to as the *Method Detection Limit* (MDL).

Limit of Quantitation (LOQ): The minimum mass or concentration of analyte that can be quantified with acceptable accuracy and precision. Limit of quantitation (or quantification) is variously defined but must be a value greater than the Method Detection Limit and should apply to the complete analytical method.

Limit Test: A type of semi-quantitative screening method in which analyte(s) has a defined level of concern. Also referred to as binary or pass/fail tests.

Linearity: The ability of the method to obtain test results directly proportional to the analyte concentration or mass within a given range.

Matrix: All the constituents of the test sample with the exception of the analyte.

Matrix Blank: A substance that closely matches the samples being analyzed with regard to matrix components. Ideally, the matrix blank does not contain the analyte(s) of interest but is subjected to all sample processing operations including all reagents used to analyze the test samples. The matrix blank is used to determine the absence of significant interference due to matrix, reagents and equipment used in the analysis.

Matrix Effect: An influence of one or more components from the sample matrix on the measurement of the analyte concentration or mass. Matrix effects may be observed as increased or decreased detector responses, compared with those produced by simple solvent solutions of the analyte.

Matrix Source: The origin of a test matrix used in method validation. A sample matrix may have variability due to its source. Different food matrix sources can be defined as different commercial brands, matrices from different suppliers, or in some cases different matrices altogether. For example, if a variety of food matrices with differing physical and chemical properties are selected, the number of sources for each food sample matrix may be one or more.

Matrix spike: An aliquot of a sample prepared by adding a known amount of analyte(s) to a specified amount of matrix. A matrix spike is subjected to the entire analytical procedure to establish if the method is appropriate for the analysis of a specific analyte(s) in a particular matrix. Also referred to as a *Laboratory Fortified Matrix*.

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Method blank: A substance that does not contain the analyte(s) of interest but is subjected to all sample processing operations including all reagents used to analyze the test samples. An aliquot of reagent water is often used as a method blank in the absence of a suitable analyte-free matrix blank.

Method Detection Limit (MDL): The minimum mass or concentration of analyte that can be statistically differentiated from analyte-free sample matrix using a specific method with acceptable certainty, though not quantifiable with acceptable precision. This is dependent on sensitivity, instrumental noise, blank variability, sample matrix variability, and dilution factor.

Method Development: The process of design, optimization and preliminary assessment of the performance characteristics of a method.

Method Validation: The process of demonstrating or confirming that a method is suitable for its intended purpose. Validation includes demonstrating performance characteristics such as accuracy, precision, specificity, limit of detection, limit of quantitation, linearity, range, ruggedness and robustness.

Method Verification: The process of demonstrating that a laboratory is capable of replicating a validated method with an acceptable level of performance.

Minimum Detectable Concentration (MDC): In qualitative analysis, an estimate of the minimum concentration of analyte that must be present in a sample to ensure at a specified high probability (typically 95% or greater) that the measured response will exceed the detection threshold, leading one to correctly conclude that an analyte is present in the sample.

Precision: Degree of agreement of replicate measurements under specified conditions. The precision is described by statistical methods such as a standard deviation or confidence limit. See also *Random Error*. Precision can be further classified as *Repeatability*, *Intermediate Precision*, and *Reproducibility*.

Qualitative Analysis/Method: Analysis/method in which substances are identified or classified on the basis of their chemical, biological or physical properties. The test result is either the presence or absence of the analyte(s) in question.

Quantitative Analysis/Method: Analysis/method in which the amount or concentration of an analyte may be determined (or estimated) and expressed as a numerical value in appropriate units with acceptable accuracy and precision.

Random error: The irreproducibility in making replicate measurements resulting from random changes in experimental conditions that affects the precision of a result. The

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distribution of random errors usually follows a Gaussian bell-shaped curve. See also *Precision*.

Range: The interval of concentration over which the method provides suitable accuracy and precision.

Reagent Blank: Reagents used in the procedure taken through the entire method. Reagent Blanks are used to determine the absence of significant interference due to reagents or equipment used in the analysis.

Recovery: Proportion of incurred or added analyte which is extracted and measured from the analytical portion of the test sample. Recovery is usually expressed as a percentage.

Reference material: A material or substance, one or more of whose property values are sufficiently homogenous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.

Reference standard: A standard, generally having the highest metrological quality available at a given location in a given organization, from which measurements are made or derived. Note: Generally, this refers to recognized national or international traceable standards provided by a standards producing body such as the National Institute of Standards and Technology (NIST).

Repeatability: Within-laboratory precision obtained under the same conditions of measurement over a short period of time.

Representative Analyte: An analyte used to assess probable analytical performance with respect to other analytes having similar physical and/or chemical characteristics. Acceptable data for a representative analyte are assumed to show that performance is satisfactory for the represented analytes. Representative analytes should include those for which the worst performance is expected. Representative analytes are used mostly for non-targeted analysis and unknown screening procedures.

Representative Matrix: Matrix used to assess probable analytical performance with respect to other matrices, or for matrix-matched calibration, in the analysis of broadly similar commodities. For food matrices, similarity is usually based on the amount of water, fats, protein, and carbohydrates. Sample pH and salt content can also have a significant effect on some analytes.

Reproducibility: Precision obtained among multiple laboratories.

Ruggedness/Robustness: The ability of a method to resist changes in test results when subjected to minor deviations in experimental conditions of the procedure.

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Ruggedness testing examines the behavior of an analytical process when subtle small changes in the environment and/or operating conditions are made and provides an indication of reliability during normal usage.

Screening Method: A method intended to detect the presence of an analyte in a sample at or above some specified concentration (target level).

Selectivity: The degree to which a method can discriminate between the analyte of interest and other components of the sample including other analytes, matrix components, and other potential interferences. Selectivity is generally preferred over the term *Specificity*. In qualitative analysis, selectivity is the ability to detect true negative samples as negative.

Sensitivity: In quantitative analysis, sensitivity is the level of instrumental response obtained per unit amount of analyte. Sensitivity is commonly defined as the slope of the calibration curve at a level near the LOQ. In qualitative analysis, sensitivity is the ability to detect true positive samples as positive.

Specificity: In quantitative analysis, specificity is the ability of a method to measure analyte in the presence of components which may be expected to be present. The term *Selectivity* is generally preferred over *Specificity*. In qualitative analysis, specificity is the ability to detect true negative samples as negative.

Spike Recovery: The fraction of analyte remaining at the point of final determination after it is added to a specified amount of matrix and subjected to the entire analytical procedure. Spike Recovery is typically expressed as a percentage. Spike recovery should be calculated for the method as written. For example, if the method prescribes using deuterated internal standards or matrix-matched calibration standards, then the reported analyte recoveries should be calculated according to those procedures.

Standard: A substance of known identity and purity and/or concentration.

Standard Reference Material (SRM): A certified reference material issued by the National Institutes of Standards and Technology (NIST) in the United States. (www.nist.gov/SRM).

Systematic error: A form of measurement error, where error is constant across trials. This may also be referred to as *Bias*.

Threshold Value: In qualitative analysis, the concentration of the analyte that is either statistically lower than the level of concern (for limit tests) or at which positive identification ceases (for confirmation of identity methods). See also *Cut-off Concentration*.

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Trueness: The degree of agreement of the mean value from a series of measurements with the true value or accepted reference value. This is related to systematic error (bias).

Uncertainty: The parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measured value.

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APPENDIX 2. Various Acceptability Criteria for Certain Performance Characteristics

A. QUANTITATIVE METHOD RECOVERY GUIDELINES

Reference: AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals

Acceptable recovery is a function of the concentration and the purpose of the analysis. Some acceptable recovery requirements for individual assays are as follows:

Concentration	Recovery limits
100 %	98-101%
10 %	95-102%
1 %	92-105%
0.1 %	90-108%
0.01%	85-110%
10 µg/g (ppm)	80-115%
1 µg/g (ppm)	75-120%
10 µg/kg (ppb)	70-125%

Reference: The Codex Alimentarius Volume 3 “Residues of Veterinary Drugs in Foods”

Suggested recovery limits for residues of veterinary drugs in foods are as follows:

Concentration Range	Acceptable Range
≤1 µg/kg	50 – 120 %
≥1 to <10 µg/kg	60 – 120 %
≥10 to <100 µg/kg	70 – 110 %
≥100 µg/kg	80 – 110 %

Reference: CVM Guidance for Industry #3, “General Principles for Evaluating the Safety of Compounds Used in Food-Producing Animals”

The acceptable range for recovery of marker residues are as follows:

Concentration Range	Acceptable Range
≥100 µg/kg (ppb)	80 – 110 %
<100 µg/kg (ppb)	60 – 110%

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B. QUANTITATIVE METHOD PRECISION GUIDELINES

Reference: AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals (excerpts from AOAC SLV Guidelines shown)

Repeatability Precision (s_r , RSD_r)

Repeatability refers to the degree of agreement of results when conditions are maintained as constant as possible with the same analyst, reagents, equipment, and instruments performed within a short period of time. It usually refers to the standard deviation of simultaneous duplicates or replicates, s_r . It is the best precision that will be exhibited by a laboratory but it is not necessarily the laboratory's typical precision.

Preferably sets of replicate analyses should be conducted at least in separate runs and preferably on different days. The repeatability standard deviation varies with concentration, C expressed as a mass fraction. Acceptable values approximate the values in the following Table or calculated by the formula:

$$RSD_r = C^{-0.15}$$

Concentration	Repeatability (RSD_r)
100 %	1 %
10 %	1.5%
1 %	2 %
0.1 %	3 %
0.01%	4 %
10 ug/g (ppm)	6 %
1 ug/g	8 %
10 ug/kg (ppb)	15 %

Acceptable values for repeatability are between $\frac{1}{2}$ and 2 times the calculated values. Alternatively a ratio can be calculated of the found value for RSD_r to that calculated from the formula designated as $HORRAT_r$. Acceptable values for this ratio are typically 0.5 to 2:

$$HORRAT_r = RSD_r (\text{found, \%}) / RSD_r (\text{calculated, \%})$$

Reproducibility Precision (s_R , RSD_R)

Reproducibility precision refers to the degree of agreement of results when operating conditions are as different as possible. It usually refers to the standard deviation (s_R) or the relative standard deviation (RSD_R) of results on the same test samples by different

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laboratories and therefore is often referred to as “between-laboratory precision” or the more grammatically correct “among-laboratory precision.” It is expected to involve different instruments, different analysts, different days, and different laboratory environments and therefore it should reflect the maximum expected precision exhibited by a method. Theoretically it consists of two terms: the repeatability precision (within-laboratory precision, s_r) and the “true” between-laboratory precision, s_L . The “true” between-laboratory precision, s_L , is actually the pooled constant bias of each individual laboratory, which when examined as a group is treated as a random variable. The between-laboratory precision too is a function of concentration and is approximated by the Horwitz equation, $s_R = 0.02C^{0.85}$. The AOAC/IUPAC protocol for interlaboratory studies [equivalent to Level 4 validation in this document] requires the use of a minimum of 8 laboratories examining at least 5 materials to obtain a reasonable estimate of this variability parameter, which has been shown to be more or less independent of analyte, method, and matrix.

In the absence of such an interlaboratory study, the interlaboratory precision may be estimated from the concentration as indicated in the following Table or by the formula (unless there are reasons for using tighter requirements):

$$RSDR = 2C^{-0.15}$$

$$\text{Or } S_R = 0.02C^{0.85}$$

Concentration, C	Reproducibility (RSDR)
100 %	2 %
10 %	3 %
1 %	4 %
0.1 %	6 %
0.01%	8 %
10 ug/g (ppm)	11 %
1 ug/g	16 %
10 ug/kg (ppb)	32 %

Acceptable values for reproducibility are between $\frac{1}{2}$ and 2 times the calculated values. Alternatively a ratio can be calculated of the found value for RSD_R to that calculated from the formula designated as $HORRAT_R$. Acceptable values for this ratio are typically 0.5 to 2:

$$HORRAT_R = RSD_R (\text{found, \%}) / RSD_R (\text{calculated, \%})$$

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Reference: Codex Alimentarius Commission, Procedural Manual, "Guidelines for the Inclusion of Specific Provisions in Codex Standards and Related Texts"

This is similar to AOAC's above except for <0.01 ppm when RSDR is 44%.

The calculated repeatability and reproducibility values can be compared with existing methods and a comparison made. If these are satisfactory then the method can be used as a validated method. If there is no method with which to compare the precision parameters then theoretical repeatability and reproducibility values can be calculated from the Horwitz equation. (M. Thompson, Analyst, 2000, 125, 385-386).

C. QUANTITATIVE METHOD ACCURACY/TRUENESS GUIDELINE

Reference: Codex Alimentarius Commission, Procedural Manual, "Guidelines for the Inclusion of Specific Provisions in Codex Standards and Related Texts"

For the evaluation of trueness, the use of certified reference materials (CRMs) is preferred. The method should provide the certified value (allowing for measurement uncertainty).

D. QUALITATIVE METHOD ACCEPTABILITY GUIDELINE

Reference: Codex Alimentarius Volume 3 "Residues of Veterinary Drugs in Foods"

Less than 5 percent false negatives and less than 10 percent false positives are expected for immunological methods intended to determine the presence or absence of a compound at some designated level of interest,

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Appendix 3. Examples of Validation Plans

A. Extension to other matrices with the same analyte(s) (Level One Emergency Matrix Extension)

This scheme represents an emergency use method extension plan for Matrix Y and Analyte X. This plan utilizes two different sources of matrix. *In cases where a representative matrix is being used to characterize a whole family of commodities, it is recommended that additional, different commodities from that family are used as “sources.”* Note that this plan is for emergency use only – the new matrix (or matrices) cannot be officially included in the scope of the method until at the minimum a Level Two Validation is performed.

Plan for Single Matrix	Matrix	Samples 1 & 2	Samples 3 & 4	Samples 5 & 6	Samples 7 & 8
Day 1	Matrix Y (Source 1)	Blank	Fortified (1/2X)	Fortified (X)	Fortified (2X)
	Matrix Y (Source 2)	Blank	Fortified (1/2X)	Fortified (X)	Fortified (2X)

Notes:

- Test portion matrices listed as Matrix Y represent 2 different commercial brands.
- Fortification level (Analyte X) represents either the action level identified for the method or two times the method LOQ.
- Fortification of each matrix can be done on the same day.

B. Extension to similar analytes in the same matrix (Level Two Validation)

A validated method can be extended to other potential analyte(s) belonging to the same chemical group. For example, a toxin method can be extended to other similar toxins. An example of the composition of a set of validation studies for method extension is shown in the following Table for analytes Y and Z in canned corn from 3-5 different sources where the method is validated for analyte A in corn

DAYS	Matrix	Analyte Y fortification levels	Analyte Z fortification levels
DAY 1	Corn 1,2,3,4 & 5	0, 1/2X, X, 2X	0, 1/2X, X, 2X
DAY 2	Corn 1,2,3,4 & 5	0, 1/2X, X, 2X	0, 1/2X, X, 2X
DAY 3	Corn 1,2,3,4 & 5	0, 1/2X, X, 2X	0, 1/2X, X, 2X
DAY 4	Corn 1,2,3,4 & 5	0, 1/2X, X, 2X	0, 1/2X, X, 2X
DAY 5	Corn 1,2,3,4 & 5	0, 1/2X, X, 2X	0, 1/2X, X, 2X

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Notes:

- i. Three - five different commercial brands of same product will be analyzed.
- ii. Fortification level 'x' is the Action level as stated in the method. In the absence of an action level, x represents two times the method LOQ.
- iii. Each sample will be analyzed at 0 fortification level and at 1/2X, X and 2X fortification level.
- iv. QC samples: The analyst will use a blank (negative control)..

C. Single matrix and single analyte (Level Two Validation):

This plan utilizes 3 different commercial brands of one matrix (M), numbered 1-3 in tables. The single matrix is being validated for a single analyte.

Plan for single matrix and single analyte	Matrix 1	Matrix 2	Matrix 3
Day 1	Blank Fortified (X)	Fortified (X) Fortified (2X)	Blank Fortified (1/2X)
Day 2	Fortified (2X) Fortified (1/2X)	Blank Fortified (1/2X)	Blank Fortified (2X)
Day 3	Fortified (1/2X) Fortified (X)	Fortified (2X) Blank	Fortified (X) Fortified (X)
Day 4	Fortified (2X) Blank	Fortified (X) Fortified (1/2X)	Fortified (2X) Fortified (1/2X)

Notes:

- i Sample matrix (M) listed as M 1-3, represents one matrix from 3 different sources of matrix.
- ii Fortification level: fortification will be at the level of concern or Action level as stated in the method (X), and at levels corresponding to 1/2X and 2X. In the absence of a defined action level, X is two times the limit of quantitation (2xLOQ) for the method.
- iii Each of 3 different sources of matrix will be analyzed 8 times (replicate analyses) over the course of experiment, two times unfortified, two times fortified at each level.
- iv. The validation will take place over a period of 4 days.
- v. Other fortification plans meeting requirements specified in note iii may be used.
- vi. QC samples: The analyst will use a blank (negative control) sample.

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APPENDIX 4. Selection of Representative Matrices

Two tools that can aid in selection of representative matrices when designing a validation protocol for a method intended to have applicability to a broad scope of products are shown below.

I – Table of Commodity Categories

Reference: EU Document No. SANCO/10684/2009, “Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed”

Vegetables, fruits and cereals

Commodity groups	Commodity categories	Typical representative commodities included in the category
High water content	Pome fruit	Apples, pears
	Stone fruit	Apricots, cherries, peaches
	Bulb vegetables	Bulb onion
	Fruiting vegetables/cucurbits	Tomatoes, peppers, cucumber, melon
	Brassica vegetables	Cauliflower, Brussels sprout, cabbage, broccoli
	Leafy vegetables and fresh herbs	Lettuce, spinach, basil
	Stem and stalk vegetables	Leek, celery, asparagus
	Forage/fodder crops	Fresh alfalfa, fodder vetch, fresh sugar beets
	Fresh legume vegetables	Fresh peas with pods, petit pois, mange tout, broad bean, runner bean, dwarf French bean
	Leaves of root and tuber vegetables	Sugar beet and fodder beet tops
	Fresh Fungi	Champignons, chanterelles
	Root and tuber vegetables or feed	Sugar beet and fodder beet roots, carrot, potato, sweet potato

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Vegetables, fruits and cereals (continued)

Commodity groups	Commodity categories	Typical representative commodities included in the category
High oil content	Tree nuts	Walnut, hazelnut, chestnut
	Oil seeds and products thereof	Oilseed rape, sunflower, cotton-seed, soybeans, peanuts, sesame etc. Oils and pastes (e.g. peanut butter, tahina) thereof,
	Oily fruits and products	Olives, Avocados and oils and pastes thereof
High starch and/or protein content and low water and fat content	Dry legume vegetables/pulses	Field bean, dried broad bean, dried haricot bean (yellow, white/navy, brown, speckled)
	Cereal grain and products thereof	Wheat, rye, barley and oat grain; maize, rice, whole meal bread, white bread, crackers, breakfast cereals, pasta
High acid content and high water content	Citrus fruit	Lemons, mandarins, tangerines, oranges
	Small fruit and berries	Strawberry, blueberry, raspberry, Black currant, red currant, white currant, grapes
	Other	Kiwifruit, pineapple, rhubarb
High sugar and low water content	Dried fruit	Raisins, dried apricots, dried plums, fruit jams
“Difficult or unique commodities”		Hops, Cocoa beans and products thereof, Coffee, Tea, Spices

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Products of animal origin

Commodity groups	Commodity categories	Typical representative commodities included in the category
Meat	Red meat	Beef, pork, lamb, game, horse
	White meat	Chicken, duck, turkey
	Fish	Cod, haddock, salmon, trout
	Offal*	Liver, kidney
	Fat from meat	
Milk and milk products	Milk	Cow, goat and buffalo milk
	Cheese	Cow, goat cheese
	Yogurt	
	Cream	
	Butter	
Eggs	Eggs	Chicken, duck, quail, goose eggs
Honey	Honey	

*Offal (liver, kidney) should be validated separately, if necessary

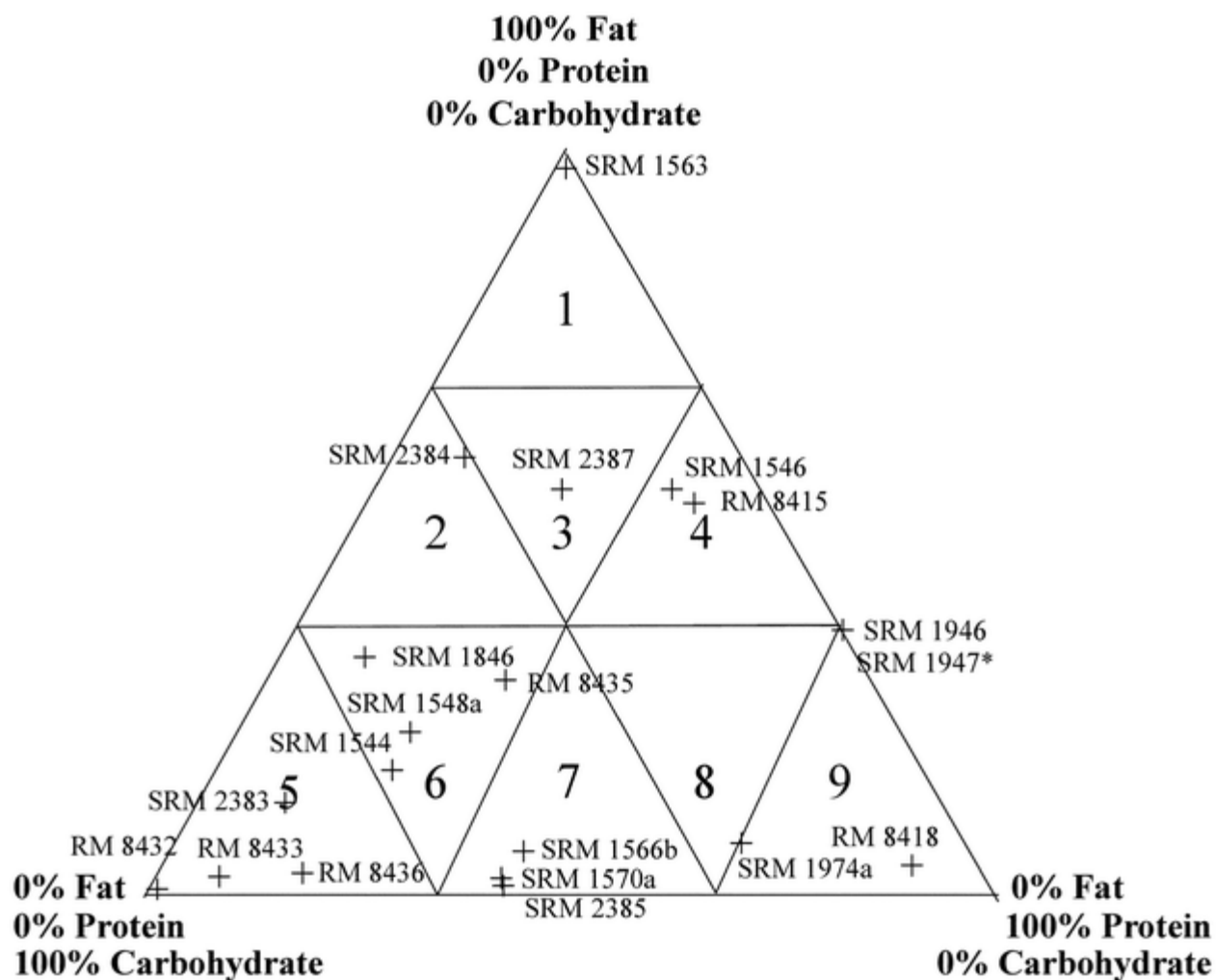
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II – AOAC Fat-Protein-Carbohydrate Triangle

Reference: K.E. Sharpless, R.R. Greenberg, M.M. Schantz, M.J. Welch, S.A. Wise, and M. Ihnat, "Filling the AOAC Triangle with Food-Matrix Standard Reference Materials", *Analytical and Bioanalytical Chemistry*, 2004, 378, 1161-1167.

The sectors in the triangle below designate varying relative fat, protein and carbohydrate content in a food product. The SRMs listed in each sector are representative of that sector and are available from NIST. The description of each SRM is provided in the table following the triangle.



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SRM or RM #	NIST Description
1563	Vitamins in coconut Oil
2384	Baking chocolate, 100% cocoa
2387	Peanut butter
1546	Meat homogenate
8415	Whole egg powder
8432	Corn starch
8433	Corn bran
8436	Durum wheat flour
2383	Baby food composite
1846	Infant formula (milk based)
8435	Whole milk powder
1548a	Typical diet
1544	Fatty acids/cholesterol in frozen diet composite
1566b	Oyster tissue
1570a	Trace elements in spinach leaves
1974a	Organics - Mussel tissue
8418	Wheat gluten
2385	Slurried Spinach
1946	Lake Superior Fish Tissue
1947	Lake Michigan Fish Tissue

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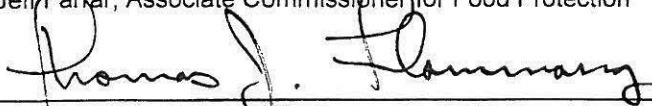
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Jeff Farrar, Associate Commissioner for Food Protection

3/8/12
Date



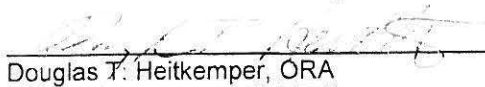
Thomas Flammang, NCJR

3.6.2012
Date



William T. Flynn, CVM

3-16-12
Date



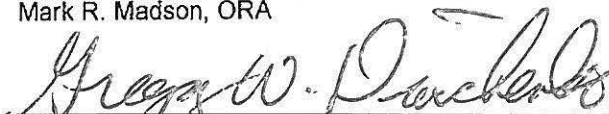
Douglas T. Heitkemper, ORA

3/6/12
Date



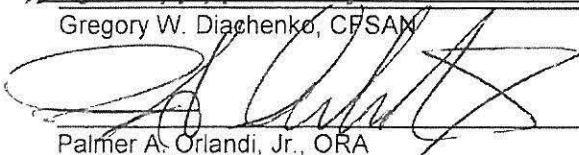
Mark R. Madson, ORA

3/8/12
Date



Gregory W. Diachenko, CFSAN

3/8/12
Date



Palmer A. Orlandi, Jr., ORA

19 MAR 12
Date



Carlos L. Peña, OC

03.14.12
Date



William B. Martin, ORA

3/8/12
Date



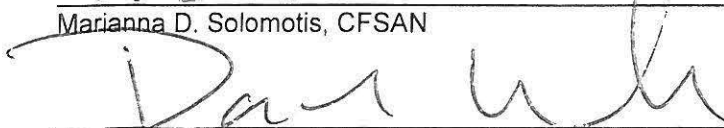
Carl Sciacchitano, OIP

3/6/12
Date



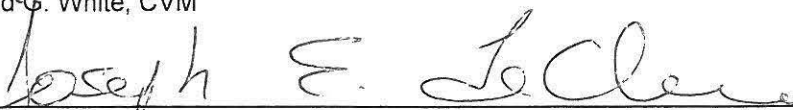
Marianna D. Solomotis, CFSAN

3/12/2012
Date



David G. White, CVM

3/8/12
Date



Donald Zink, CFSAN

3/8/12
Date

For Don Zink